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NOTES ON METHODS OF LABORATORY TECHNIQUE FROM THE ZOOLOGICAL LABORATORY, UNIVERSITY OF MICHIGAN

The writer presents the following methods of laboratory technique knowing full well that little originality can be claimed for them but with the feeling that they have been sufficiently tried to prove their worth.

Neutral red (Neutral Roth) as an indicator of the reaction of the digestive fluids of Protozoa.—That neutral red may be used as an indicator of the reactions of the digestive fluids of Protozoa has been known for a considerable time but its use as such is not as general as it should be. For this purpose freshly made solutions of the dye in the proportion of 1 : 10000 to 1 : 100000 in distilled water should be used. A few drops of the solution should be drawn under the cover-glass or one or two drops of it should be added to the water on the slide containing Protozoa before placing the cover-glass. Examination should be made at once because the reaction is secured within a few minutes. In the presence of alkalies neutral red becomes yellow while in the presence of acids it becomes red. Used in this manner the stain does not injure the Protozoa appreciably. This test is readily made even by a beginning student and it is easily interpreted by him. In this laboratory it has been used in the study of Paramecium in order to introduce a little idea of the physiology of Protozoa. It should be further stated that after the digestive fluid in the food vacuoles becomes well stained the mouth and gullet are more readily seen than in the untreated specimens.

To demonstrate the difference in function of the skin glands of the frog.—It has been the usual experience that ordinary methods of fixation and staining of the skin of the frog do not yield preparations which readily show differences in function of the poison and mucus glands. Hæmatoxylin and eosin usually reveal only structural differences not differences in secretions elaborated. After numerous trials the following method was found to give best results: Pieces of skin including the dorso-lateral dermal plicæ are removed from a pithed frog, tied to a slide flesh side out and fixed over night in Bouin's picro-aceto-formol mixture. Ten micron sections from this material are stained for a few minutes in a 0.1%

solution of thionin in distilled water, washed for a few seconds to a minute in distilled water, and briefly stained in a 0.5% solution of erythrosin. The section must then be dehydrated rapidly, cleared in xylol and mounted in balsam. By this method all nuclei are stained blue, the contents of the small glands and the granules within the cells of these glands are stained blue to reddish-purple. Since thionin is a specific mucin stain these glands are shown to be mucus glands. While the nuclei of the cells of the large glands are stained blue the cells are either unstained or are pink; the secretion which appears in the lumen of the gland as globules of varying sizes is stained a brilliant reddish pink or red tinged with yellow. Thus these glands are shown at once to be different in function from the mucus glands, and the inference is that they are poison glands. With such striking colorations the two kinds of glands are distinguished at a glance. By this method the muscles surrounding the glands are stained pink, the stratum compactum another shade of pink, blood cells pink or coppery red with blue nuclei. Boundaries of epidermal cells are clearly shown.

Fixation in Gilson's fluid followed by these stains gives fair results but not as good as by the method outlined above. Unfortunately sections stained by this method are not colored uniformly from end to end, nor are different slides stained alike, nor is it possible to turn out slides by wholesale when using these methods because each slide must have individual treatment. Slides so stained, however, show many features not well differentiated by hæmatoxylin stains in combination with other stains.

In the absence of thionin toluidin blue (Toluidin-Blau) used in the same fashion gives almost the same results. Kresylectviolett has not been tried on this tissue but it should be pointed out that it is an excellent mucin stain.

A method for staining the cephalic glands of immature trematodes.—In an attempt to work out the relations of the cephalic glands to their ducts in some minute immature trematodes a number of aniline dyes, carmine, hæmatoxylin stains were tried. The material had been fixed in formalin of unknown strength. Hæmatoxylin which was first tried for toto preparations and sections gave altogether erroneous ideas of relationships of the glands to the ducts and wrong ideas of the ducts because of over-staining and too diffuse staining. Carmines were hardly more successful. Out of a number of aniline dyes solutions of thionin and of toluidin blue, ca 1% in distilled water, gave excellent results either for toto mounts or for sections. Whole specimens or sections were stained about ten minutes in either of these stains, differentiated by washing in distilled water, dehydrated and finally cleared in xylol and mounted in balsam. The length of time required for differentiation of the stain in the whole worms was several minutes while a few seconds or at most a minute sufficed for the sections. Sections were sometimes given a second stain with erythrosin which served to stain muscles but did not aid in the study of the glands and the ducts. The contents of the glands and of their ducts when stained with thionin or toluidin blue are blue or purple in color and are seen to consist of great numbers of small globules. In this case the indications are that this material is mucin. The walls of the glands and ducts remain unstained. As a result of this fact two ducts which lie very closely together and which appear as one when stained with hæmatoxylin or carmine are shown as separate structures. Certain other structures are shown fairly well. The method seems to be inapplicable to toto preparations of large trematodes.

To fasten the wax to the bottoms of dissecting pans.—The usual method of fastening the wax in a dissecting pan is to have strips of tin or other metal soldered or riveted to the ends or to the bottom of the pan and so projecting that the melted wax will run under the strips and hardening thus the mass is held in place. This method does very well for tin or copper pans but if it is desired to use enameled pans it is not satisfactory. A method that is applicable to any kind of a pan is to use 'BB' shot or bits of granulated lead or zinc to weight down the wax. At the time of pouring in the melted wax the shot or the bits of granulated metal are placed in the corners of the pan. When cooled the weight of the metal holds the wax in place. In case the wax becomes loosened it can be readily removed from the pan, melted and repoured. When metal strips are used in the old method the wax must be melted in the pan and there is danger of opening up the seams in soldered pans.

Celluloid-acetone cement.—A very useful laboratory cement may be made by adding scraps of celluloid to acetone in which it quickly dissolves. Experience will soon teach the proper proportions. Do not make it too thick. Since acetone evaporates quickly the cement must be kept covered and the work must be rapidly done. In our laboratory it finds many uses, e. g., in mending small broken bones, cementing glass to glass or to many other substances, where a waterproof cement is desirable. In mending small broken bones with this cement it is advisable to run some of the cement into the cavity, put pieces together, cover with a thin layer of the cement on the outside. If a strong joint is necessary chop up some cotton into short bits and work it into the cement as it is used. For the mending of bones the transparent celluloid should be used to make the cement but for other purposes it may be desirable to use colored celluloid. The writer has seen this cement so skillfully used on broken bones that very close scrutiny was required to discover the fracture. Since this cement is dissolved or at least softened by strong alcohols it cannot be used to mend things that are to be stored in alcohol.

To render visible lines milled on laboratory apparatus.—The millings on apparatus that has been in use for some time usually becomes indistinct. To render it again visible polish the surface with a good metal polish and then fill the milled lines and figures with 'Shinola' shoe polish, allow to dry and then rub off that portion which remains on the plain surface. The lines will be filled with the blacking. This material being neutral in reaction will not injure the metal.

To revive or to keep going cultures of Ameba.—Ameba cultures made after the method of Jennings, i. e., by allowing plant materials collected from ponds or streams to decay in pond or stream water usually begin to fail soon after they have begun to yield Ameba in abundance. Such cultures may be kept going or, even after all amebæ have apparently disappeared, they may be made to yield in abundance if a small quantity of sterile hay be added at intervals of a few days. Probably any clean hay would do as well but if sterile hay is used there is no danger of introducing other encysted Protozoa.

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